

Spotlight on structural genomics

Structural genomics refers to the high-throughput endeavor of solving three-dimensional protein structures by X-ray crystallography, NMR and modeling. Over the past decade, structural genomics consortia have expressed, purified and collected structural information for thousands of proteins as well as developed many new methods to streamline the pipeline. In a Commentary, consortia members of the one of the largest efforts, the US-based Protein Structure Initiative (PSI), describe the current state of the art in structural genomics and the specific mission, achievements and future goals of the PSI. In a Review, structural genomics researchers from all over the world share their collective expertise to develop a consensus strategy for tackling the challenge of protein expression and purification. In addition, a Perspective explores the fine art of protein crystallization, and the Technology Feature describes the development and use of technology by the PSI-funded Joint Center for Structural Genomics.

Commentary p129, Review p135, Perspective p147, Technology Feature p203

Worm genome sequencing with short sequence reads

The speed and throughput of next-generation sequencing is impressive, but, because these technologies only produce short sequence reads, their suitability for applications such as whole-genome resequencing and variant discovery still needs to be determined. In an effort to do so, Mardis and colleagues show that they could assemble the *Caenorhabditis elegans* genome from sequences generated with Solexa technology by aligning the reads to the reference genome with the aid of new computational tools. Similarly, comparing short sequence reads from a related *C. elegans* strain allowed them to discover genome-wide polymorphisms such as single-nucleotide polymorphisms and small indels.

Article p183

Tagging endogenous proteins

Antibodies are fantastic research tools for studying proteins, but unfortunately high-quality antibodies are not available to every protein. Although overexpressing recombinant proteins tagged with an epitope to a generic antibody can circumvent this problem, overexpression may be undesirable for

certain applications. Wang and colleagues developed a good alternative by using homologous recombination between an endogenous locus and an adeno-associated virus to insert an epitope tag at a predefined genomic locus. This knock-in strategy allowed the analysis of endogenous proteins by any technique that requires antibodies, from immunofluorescence to chromatin immunoprecipitation.

Brief Communication p163

Blood monitoring of *in vivo* processes

Luciferase is a commonly used reporter *in vitro* and *in vivo*, detected in the latter case by bioluminescence imaging with a charge-coupled device (CCD) camera. Now, Tannous and colleagues use a secreted luciferase from the marine copepod *Gaussia princeps* (Gluc) as a simple, rapid, sensitive and quantitative *ex vivo* reporter for *in vivo* processes. Gluc expressed by cells within the mouse body is secreted into the blood of the animal and can report on cell number in an assay using small volumes of blood and a standard luminometer. This simple test can be used to monitor tumor growth and therapy as well as gene transfer by viruses and will be a useful complement to *in vivo* bioluminescence imaging.

Brief Communication p171

Single molecules come alive

Methodological shortcomings have limited most single-molecule studies to isolated molecules and fixed cells.

Two reports in this issue describe methods that overcome some of these limitations. Lippincott-Schwartz and colleagues

combined photoactivated localization microscopy (PALM) with single-particle tracking to track thousands of membrane proteins in live cells. In this work a total internal reflection fluorescence (TIRF) microscope that selectively illuminates only the cell membrane lying against the microscope slide constrains the imaging to this area of interest but prevents analysis of other regions. Tokunaga and colleagues describe a simple microscope adaptation they call highly inclined and laminated optical sheet (HILO) microscopy that allows single-molecule imaging in an illuminated plane inside a cell. These two reports promise to open new windows for the observation of single molecules in live cells.

Brief Communication p155, p159, News and Views p133

